NTP Research Concept: 2',2'''-Dithiobisbenzanilide

Project Leader
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Nomination Background and Rationale
2',2'''-Dithiobisbenzanilide (DBBA) was nominated by the National Cancer Institute (NCI) for genotoxicity and metabolism studies based on the lack of these data and suspicion of toxicity based on the structure of the chemical (http://ntp.niehs.nih.gov/go/29287). DBBA is a high production chemical with potential for occupational and consumer exposure. The major use for DBBA is as a peptizing agent (plasticizer) for natural and synthetic rubber. Peptizers reduce viscosity of rubber, thus allowing the material to be shaped and molded into final end products. DBBA is used in the manufacture of tires and other solid rubber goods, e.g. belts, cables, hoses, as well as in the rubber components of diverse equipment and products used commercially and by consumers. Rubber industry workers are potentially exposed to DBBA by the inhalation and dermal routes during the compounding and mixing process. The general population may be exposed to small amounts of DBBA that leach from consumer products, such as rubber and latex gloves, and/or are released into the environment during the manufacture or disposal of DBBA-containing products. DBBA is approved by the FDA as a plasticizer for use in materials that may come into contact with food during manufacture, processing, and packaging; and DBBA has been cited in some U.S. patents for use in pharmaceutical formulations. A structurally similar chemical, 2,2'-dithiobis-4'-(sulfamoyl)benzanilide, has been shown to have antiretroviral activity in lymphocyte-derived CEM cells. No standards or guidelines have been set by NIOSH or OSHA for exposure levels of DBBA in the workplace, nor is DBBA currently regulated as a hazardous substance in the U.S. DBBA has been classified as an environmentally hazardous substance and as an aquatic toxicant in Europe, based on detection of the chemical in wastewater discharged from rubber processing sites and acute toxicity data from aquatic organisms, including fish. The acute toxicity in mammals appears to be low. The reported oral LD_{50} of DBBA in rats was > 4 g/kg and the dermal LD_{50} was 10 g/kg in rabbits. There are no subchronic or carcinogenicity studies in animals or epidemiological studies of DBBA in humans reported in the literature. Increased incidence of cancer and contact dermatitis has been reported among workers in the rubber industry; however, health evaluations generally are unable to identify the specific causative agent(s). DBBA is a potential skin and respiratory irritant and may produce skin sensitization. The chemical was found to be a medium-strong sensitizer when applied in pure form to the skin of male guinea pigs. There are no reported studies for mutagenicity or metabolism of DBBA.

Key Issues
The extent of exposure to humans and the toxic potential of DBBA have not been adequately determined, although production of the chemical has increased substantially within the past few years (≤ 0.5 million to >1 million lbs/year from 1994 to 2002). Therefore, the NCI has identified the need for a complete toxicological characterization of DBBA, including subchronic studies in animals, mutagenicity studies, identifying *in vivo* metabolites, and determining the levels of DBBA and its degradation products in the environment. The latter information is essential for determining the relevance of exposure to DBBA and its degradation products to the general population, but is beyond the scope of work conducted by the National Toxicology Program

(NTP). A key issue identified for initial investigation by the NTP is to determine the potential reactivity of DBBA. Sensitization studies in guinea pigs indicate that DBBA is reactive in skin and may undergo metabolism in the tissue. However, these results do not establish the extent of dermal absorption or systemic exposure. Further, the mutagenic potential of DBBA or its potential for metabolism to mutagenic or reactive metabolites is unknown. It has been speculated that DBBA may undergo metabolism to benzanilide or benzamide derivatives possessing biological activity.

Proposed Research Program

The main goals of this research project are to investigate the *in vivo* fate of DBBA and to evaluate the mutagenicity of DBBA in a standard battery of genotoxicity tests. These studies are considered high priority for toxicological characterization of the chemical. The specific aims of the research project are to:

1. Determine the *in vitro* mutagenic potential of DBBA. Data from these studies may also be useful in characterizing the potential for DBBA to form reactive metabolites.
2. Determine the potential for dermal absorption of DBBA from human and/or rodent skin, *in vitro*.
3. Conduct *in vitro* metabolism studies using unlabeled DBBA in human and/or rodent liver preparations.
4. Determine bioavailability and characterize metabolism of radiolabeled DBBA in rodents by both the oral and dermal routes, if needed.

Significance and Expected Outcome

DBBA is the preferred peptizing agent for use in natural and synthetic rubber and has recently become a high production chemical. Consequently, the potential for human exposure has increased; however, the toxicity of DBBA has not been adequately characterized. The proposed research project would determine the potential for mutagenicity, absorption, and metabolic activation of DBBA. Results from this work would provide data critical for determining the potential toxicity of DBBA and would be used to determine the need for subchronic and long-term toxicity studies in rodents. Ultimately, these studies would provide data used to characterize risks associated with exposure to DBBA and to determine safe exposure limits for workers and consumers.

References and Supporting Materials

Domagala, J.M. *et al*. (1997) A new class of anti-HIV-1 agents targeted toward the nucleocapsid protein NCp7: The 2,2'-dithiobisbenzamides. *Bioorganic and Medicinal Chemistry*, **5**, 569-579.

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